

What is claimed is:

1. A method for a long-term culture of avian spermatogonial stem cells, which comprises the steps of:

(a) preparing an avian testis;

5 (b) isolating a population of testicular cells from said avian testis; and

(c) culturing said avian spermatogonial stem cells in said population of testicular cells on a feeder cell layer in a medium containing a cell growth factor.

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2. The method according to claim 1, wherein said step (b) is carried out by treating said avian testis with collagenase, trypsin or its mixture.

15 3. The method according to claim 2, wherein said step (b) is carried out by treating said avian testis with a mixture of collagenase and trypsin.

4. The method according to claim 1, wherein said feeder cell
20 is fibroblast, gonadal stroma cell, testicular stroma cell or mouse STO cell.

5. The method according to claim 4, wherein said feeder cell is gonadal stroma cell or testicular stroma cell.

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6. The method according to claim 5, wherein said feeder cell is gonadal stroma cell.

7. The method according to claim 1, wherein said cell growth factor is a growth factor selected from the group consisting of fibroblast growth factor, insulin-like growth factor-1, stem cell factor, glia-derived neurotrophic factor and their combination.

8. The method according to claim 1, wherein said medium further comprises a differentiation inhibitory factor.

9. The method according to claim 8, wherein said differentiation inhibitory factor is leukemia inhibitory factor.

10. The method according to claim 1, wherein said medium comprises a supplement containing a mixture of fibroblast growth factor, insulin-like growth factor-1 and leukemia inhibitory factor.

11. The method according to claim 1, wherein said medium further comprises a serum and an antioxidant.

12. The method according to claim 1, wherein said culturing is carried out at about 37°C.

13. The method according to claim 1, wherein said avian species is a chicken, a quail, a turkey, a duck, a goose, a pheasant or a pigeon.

14. The method according to claim 1, wherein after step (c) said process further comprises the step of identifying the avian spermatogonial stem cells.

5 15. The method according to claim 14, wherein said identification is carried out by (i) PAS (Periodic Acid Shiff's) staining, (ii) STA (*Solanum tubersum* agglutinin) staining, (iii) a staining with $\alpha 6$ -integrin antibody, (iv) a staining with $\beta 1$ -integrin antibody, (v) a staining with anti-SSEA-1 antibody, (vi) a staining with anti-SSEA-3 antibody,
10 (vii) a staining with anti-SSEA-4 antibody, (viii) DBA (*Doliclos biffirus* agglutinin) staining or (ix) their combination.

15 16. A population of avian spermatogonial stem cells comprising avian cells expressing characteristics of a spermatogonial stem cell.

17. The population of avian spermatogonial stem cells
20 according to claim 16, wherein said characteristics of a spermatogonial stem cell is a positive reaction to (i) PAS (Periodic Acid Shiff's) staining, (ii) STA (*Solanum tubersum* agglutinin) staining, (iii) a staining with $\alpha 6$ -integrin antibody, (iv) a staining with $\beta 1$ -integrin antibody, (v) a staining with anti-SSEA-1 antibody, (vi) a staining with anti-SSEA-3 antibody, (vii) a staining with anti-SSEA-4 antibody,
25 (viii) DBA (*Doliclos biffirus* agglutinin) staining or (ix) their combination.

18. The population of avian spermatogonial stem cells according to claim 16, wherein said population of avian spermatogonial stem cells is prepared in accordance with any
5 one of claims 1-15.

19. A method for producing a transgenic ave, which comprises the steps of:

- 10 (a) transferring a foreign gene to the population of avian spermatogonial stem cells according to any one of claims 16-18;
- (b) transplanting said population of avian spermatogonial stem cells into a testis of a recipient; and
- 15 (c) producing a progeny from said recipient to produce the transgenic ave.